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POLYNUCLEAR AROMATIC HYDROCARBONS IN ESTUARINE AND NEARSHORE ENVIRONMENTS

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Abstract: Polynuclear aromatic hydrocarbons (PNAH) are ubiquitous and are found in waters, soil, and bottom sediments throughout the world, although the concentrations are higher in industrialized locations. Interest in these compounds is generated by their carcinogenic and mutagenic properties. Although biosynthesis and petroleum spillages may be sources for PNAH's in localized areas, the major source of these compounds is pyrolytic. They tend to degrade by photo and biological oxidation. Like other hydrocarbons, they accumulate in most seafood, especially in bivalves. Generally, initial depuration is rapid; however, PNAH's might persist for years in some species.

Introduction

Polynuclear aromatic hydrocarbons (PNAH) are among the oldest known carcinogens. They were identified as the active carcinogens associated with soot which caused the first occupationally related cancer known as chimney sweep disease. They are ubiquitous and found in waters, soil, and bottom sediments throughout the world, although apparently in greater abundance in industrialized locations. Because of their carcinogenic, mutagenic, toxic, and ubiquitous nature it is important that the impact of these compounds on estuarine environments and ultimately on man be established.

In this review we will discuss primarily the aromatic compounds with more than two rings. A number of good review articles relating to this subject have been published and should be referred to: Suess, 1968, 1970; GESAMP, 1977; Blumer and Youngblood, 1975; Zobell, 1971; Hites, 1976; Andelman and Snodgrass, 1974. This review attempts to update briefly all the work on this subject and point toward some new conclusions.

Chemical and Physical Properties

PNAH's, typical of those found in the marine environment, are listed in Figure 1. Both the parent compounds listed and alkyl derivatives, containing

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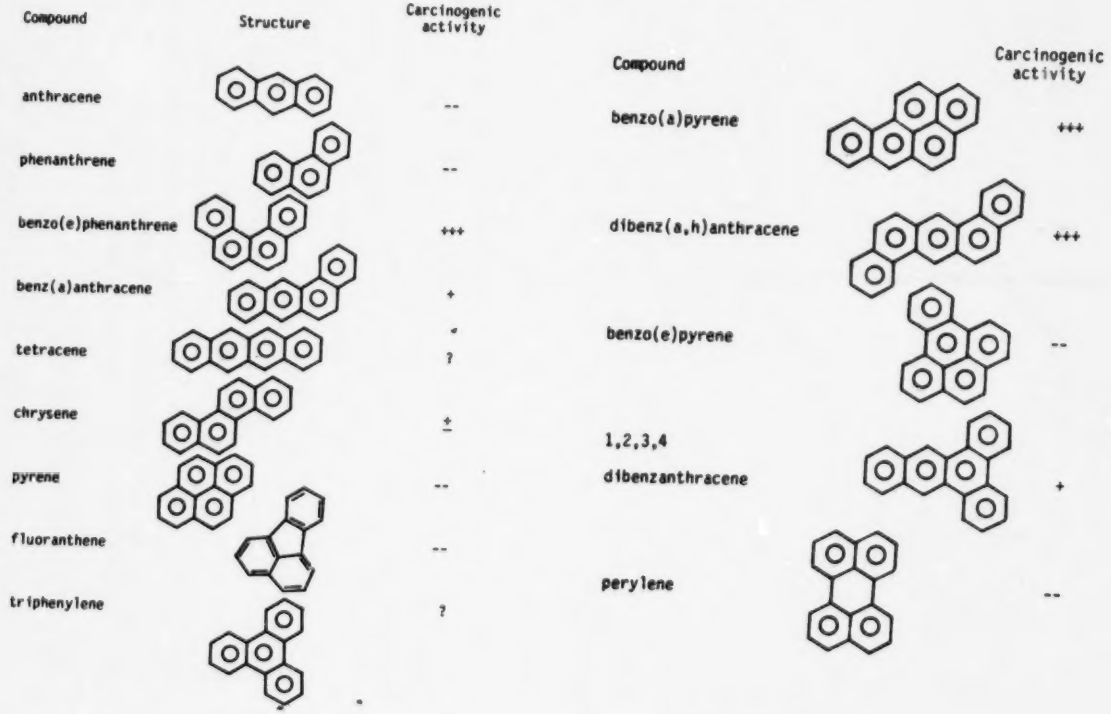
Figure 1. Typical PNAH's found in the marine environment.



up to fifteen carbon atoms in the alkyl groups, have been isolated from estuarine sediments (Blumer and Youngblood, 1975; Youngblood and Blumer, 1975; Hites and Biemann, 1975; Giger and Blumer, 1974; Hase and Hites, 1976b). As shown in Figure 1, PNAH's are planar, condensed, multiring structures which exhibit many of the chemical and physical properties of one-and two-ring aromatic compounds, i.e., they absorb ultraviolet light, are oxidized by ozone, chlorine and ultraviolet light, and undergo electrophilic substitution in specific positions on the ring, depending on the structure and substituents present (Clar, 1964). PNAH's are potent mutagens and carcinogens, others are less active or suspected carcinogens. Some PNAH's, especially those of biological origin, are probably not carcinogens.

Carcinogenicity

No clear relationship between the electronic structure and carcinogenic activity of PNAH exists, although a hypothesis has been proposed (Pullman and Pullman, 1955).

In most, if not all, cases it appears that PNAH's have to be metabolically activated by tissue microsomes before they become biologically active (Miller and Miller, 1971). The activation mechanism occurs by hydroxylation or production of unstable epoxides of PNAH's which damage DNA and thus initiate the carcinogenic process (Ames *et al.*, 1972). It is well established that fish and, to a much lesser extent, crustaceans possess the enzymes necessary for activation. In contrast, it appears that mollusks lack the metabolizing enzymes and therefore, theoretically, should not activate PNAH's (Philpot *et al.*, 1976; Carlson, 1972; Lee *et al.*, 1972). However, very recently Robert Anderson at the Sloan Kittering Institute found evidence of low-level activation by shellfish (pers. commun.). Other invertebrates have a greatly reduced ability to metabolize hydrocarbons.

Hueper (1963), Yevich and Barszcz, (1976) and more recently Brown *et al.*, (1977) have demonstrated an increased incidence of neoplasia in the soft shell clam, *Mya arenaria*, from several oil-contaminated areas. It is tempting to speculate that PNAH's are the causative agents, except for the fact that shellfish have reduced enzyme-activating ability. A direct "cause and effect" relationship has not been established. The possibility exists that activation is not necessary in these animals or that certain benthic microorganisms might perform the necessary transformation. Other disturbing factors are that measurements of hydrocarbon body burden do not correlate well with neoplasia incidence (R. Brown, pers. commun.); and secondly, in spite of intensive efforts, the field observations of neoplasia have not yet been reproduced in the laboratory.

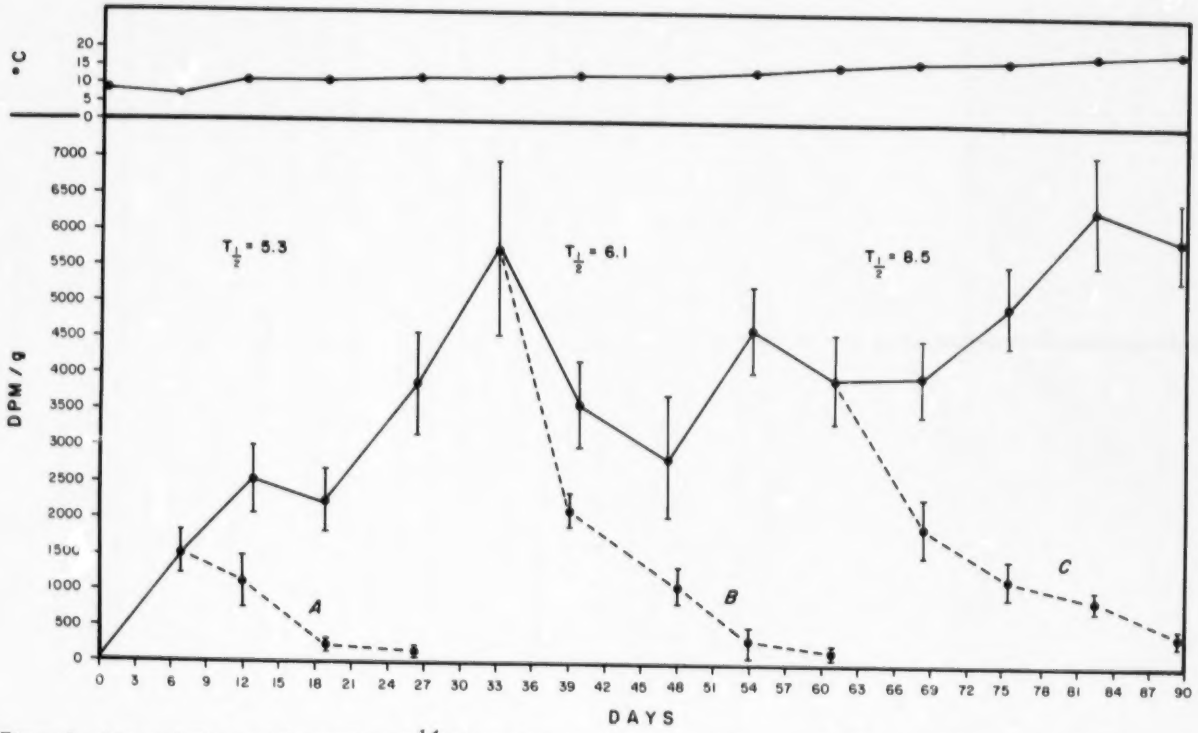


Figure 2. Accumulation and depuration of ^{14}C labelled benzo(a)pyrene in Mya arenaria. *Curves A, B, and C indicate depuration starting at three different accumulation time periods.*

Accumulation and Depuration

The accumulation of PNAH's in bivalves is considerable; for example, reported values range from non-detectable to over 1,675 μg/kg, from an industrial polluted area (Jackim and Wilson, 1977). Similarly, benzpyrene concentrations of up to 150 μg/kg have been reported in shellfish by GESAMP (1977). Since most of the carcinogenic hydrocarbons arise anthropogenically, it is not surprising that animal tissue concentrations fluctuate widely depending on the location from which the animals were obtained.

Fish apparently have the ability to avoid high concentrations of oil and other hydrocarbons. Nevertheless, benzpyrene concentrations of fifteen and sixty-five μg/kg were reported for cod from Greenland and sardine from Naples, respectively (Suess, 1970). Neoplasia have been reported in fish caught in waters contaminated by fuel oil or oil refinery wastes (Russell and Kotin, 1957). It is probable that PNAH's play a role in fish tumor development; however, the incidence of such tumors is likely to be too small to have much ecological significance.

In general, marine animals accumulate and depurate PNAH's much like other hydrocarbons, even though the rates for specific compounds differ somewhat. Alkylnaphthalenes followed by naphthalene accumulate to the greatest extent (Anderson *et al.*, 1974). The rate of PNAH accumulation in bivalves is also dependent upon water concentration and species type. Neff (1975) demonstrated that clams accumulate hydrocarbons more slowly than oysters.

It appears that most bivalves eliminate PNAH's rapidly, since PNAH's usually possess a biological half-life of between two and sixteen days (Jackim and Wilson, 1977). The only detailed report showing little or no depuration in bivalves is one concerning *Mercenaria mercenaria* by Boehm and Quinn (1977). This probably represents a different mechanism of elimination peculiar to this species, although other factors such as technical failure to resolve biogenic hydrocarbons or contamination could lead to this interpretation. Blumer, Souza, and Sass (1970) also reported on slow depuration in oysters. More recent work with oysters does not substantiate Blumer's findings (Neff, 1975). Stegeman and Teal (1973) demonstrated that about 90 percent of the accumulated hydrocarbons in oysters is released within two weeks of depuration, and the remaining 10 percent is released very slowly, suggesting that traces might remain indefinitely.

The turnover rate of hydrocarbons in fish and crustaceans is greater than in mollusks, due partly to the animals' ability to metabolize these compounds. A hypothesis was proposed, stating that with increased time of accumulation, hydrocarbon depuration rates decrease because the material becomes incorporated in slow turnover compartments. It was demonstrated, however, that increased accumulation time produced only a slight decrease in depuration rate in *Mya arenaria* (Jackim and Wilson, 1977) (Figure 2). It is still possible, however, that long-term chronic exposure as found in natural situations might

decrease depuration rates and explain Blumer's observations. Benzpyrene and presumably other PNAH's tend to concentrate in the viscera and mantle; the abductor muscle and siphon accumulate only a small percentage of the total contaminant (Neff and Anderson, 1975; Jackim and Wilson, 1977).

Biomagnifications and Health Risk

Because of the relatively rapid degradation and depuration of PNAH's in most animals and low absorption by the intestinal tract in higher animals, it is unlikely that any significant biological magnification of PNAH concentration occurs up the food chain. The question of potential carcinogenic or mutagenic risk to man as a consumer of marine food does arise and is impossible to evaluate fully at this time because of the many unknowns concerning carcinogenicity. Among these are the unknowns concerning threshold levels, synergistic or co-carcinogenic effects and cellular recovery processes.

Looking at benzpyrene, we see that *Mya arenaria* from highly contaminated locations have upper values between 30 and 150 μg/kg. From clean areas the concentration generally drops to levels between trace and 1 to 5 μg/kg (Mix *et al.*, 1977). Comparing these levels to that of other common foods, we find the non-polluted levels not extraordinarily high. For comparison, charcoal broiled meats range from 2.6 to 11.2 μg/kg, smoked ham or sausage 0.02 to 14.6 μg/kg, cooked fish 0.9 μg/kg, bread 0.1 to 4.1 μg/kg, margarine 0.2 to 6.8 μg/kg, and smoked fish 0.3 to 60 μg/kg (GESAMP, 1977). We must remember that shellfish are not eaten in the quantities that some of the other foods mentioned above are, and also that they are generally not harvested from polluted areas. On the basis of existing evidence it appears that moderate consumption of shellfish from non-polluted waters presents little significant risk from PNAH's at this time. Shellfish from polluted areas might present risks from PNAH's as well as other pollutants. If necessary, a depuration period of several weeks in clean water should greatly decrease the PNAH levels in most seafood, with the possible exception of *Mercenaria mercenaria*. Fish and crustaceans present less of a problem than shellfish.

Distribution and Transportation

Polynuclear aromatic hydrocarbons are ubiquitous and are found in water, air, soil, and marine and lake sediment samples in small but detectable concentrations. In general, concentrations of PNAH are higher in samples taken near industrialized areas. It is well documented that PNAH's are found in marine sediment (Gearing *et al.*, 1976; Tissier and Oudin, 1973; Farrington and Quinn, 1973; Mallet *et al.*, 1963; Bourcart and Mallet, 1965; Lalou *et al.*, 1962; Bourcart *et al.*, 1961; Mallet *et al.*, 1960; Mallet and Le Theule, 1961; Piccinetti, 1967; Scarratt and Zitko, 1972; Vandermuelen *et al.*, 1977; Giger and Blumer, 1974; Hites and Biemann, 1975; MacLeod *et al.*, 1976; Orr and Grady,

1967; Aizenshtat, 1973), in water samples (Brown *et al.*, 1973) and manganese nodules (Thomas and Blumer, 1964).

Numerous routes exist for the transport of PNAH's into the marine environment. The high concentrations of PNAH's present in incomplete combustion products from vehicle exhaust and industrial smoke represent a major contributor of PNAH's to the marine waters, via atmospheric fallout. Inputs of sewage and industrial effluents, oil refinery effluent, urban runoff, shipping and harbor oil spillages, and harbor dredge spoils to estuarine and coastal waters contribute to the high concentrations of PNAH's found in localized areas.

The wide distribution of PNAH's in marine waters and sediment may be surprising in light of their limited solubility in water. Though the water solubility of PNAH's, especially those containing more than three rings, has been estimated at less than 10^{-10}M (Radding *et al.*, 1976), McGinnes and Snoeyink (1974) showed experimentally that 1,2 benzanthracene and 3,4 benzpyrene did not form a true solution with water but rather were present as a particulate suspension. Therefore the transportation and distribution of PNAH's in the marine environment is governed either by the movement of water containing solubilized PNAH's or by the movement, settling, and resuspension of particles to which PNAH's adhere. Radding *et al.* (1976) stated that, since the concentration of detergents in most coastal waters is generally less than that necessary for micelle formation, this mechanism of transport is of little importance to the marine environment. This may not be the case in estuarine and coastal water where the input of urban runoff, domestic sewage, and fulvic and humic acids may contribute high concentrations of detergents and surfactants which can solubilize otherwise insoluble organic compounds such as PNAH's (Schnitzer and Khan, 1972).

Laboratory studies have shown that PNAH's adsorb onto suspended estuarine material (Lee, 1977), kaolinite (McGinnes and Snoeyink, 1974), and calcite (Suess, 1968), and the movement of these contaminated particles is believed to be most important in the transport of PNAH's in the marine environment (Lee, 1977; Radding *et al.*, 1976; Suess, 1968; McGinnes and Snoeyink, 1974). Though UV and fluorescent identifications of specific PNAH's have been reported, the presence of numerous alkylated-homologous series of PNAH's in extracts of marine sediment (Hites and Biemann, 1975; Hase and Hites, 1976b; Youngblood and Blumer, 1975; Blumer and Youngblood, 1975; Giger and Blumer, 1974) can lead to bathochromic shifts in UV spectra and spectral overlap (Clar, 1964; Giger and Blumer, 1974). Due to the spectral problems with these complex mixtures, some researchers have stated that the assignment of specific peaks or areas of UV and fluorescent spectra to specific compounds is tenuous (Hargrave and Phillips, 1975). Others suggest that only through the combination of column chromotographic, GC/MS techniques, and UV and fluorescent spectrophotometric methods, can complete identification of compounds from complex mixtures be accomplished (Giger and Blumer, 1974; MacLeod *et al.*, 1976).

Sources

The ubiquitous distribution of PNAH's has led to a dispute regarding the source of these compounds. Niaussat (1970), Mallet and Tissier (1969), Niaussat and Attenwalder (1969), Zobell (1959, 1971), and Borneff *et al.*, (1968), to mention a few, reported endogenic synthesis of PNAH's by plants, algae, and bacteria; their findings have been disputed by researchers who believe the origins of these compounds were either pyrolytic (Hase and Hites, 1976a, b; Giger and Blumer, 1974; Youngblood and Blumer, 1975) or a result of petroleum pollution. Grimmer and Dueval (1970) used elaborate precautions to eliminate contamination by air-borne PNAH's and found that higher plants did not synthesize PNAH's. Hase and Hites (1976a) found that extracts of both test bacteria and reagent blanks contained comparable amounts of PNAH's (anthacene and/or phenanthrene, pyrene, chrysene, perylene). They concluded that bacteria are effective bioaccumulators of PNAH (by a factor of 500). In view of these findings, the results of earlier researchers (Mallet and Tissier, 1969; Niaussat and Attenwalder, 1969; Niaussant, 1970; Zobell, 1959, 1971) could be interpreted as bioaccumulation rather than biosynthesis. The use of GC/MS instrumentation (Youngblood and Blumer, 1975; Blumer and Youngblood, 1975; Hites and Biemann, 1975; Hase and Hites, 1976a; Giger and Blumer, 1974) demonstrated the great variety of PNAH and their alkyl homolog derivatives, the predominance of unsubstituted PNAH's and the presence of sulfur-containing compounds (benzothiophene, dibenzothiophene) in extracts from marine and marsh sediments. The likelihood that organisms could produce a similar complex mixture under a variety of environment conditions seemed remote (Youngblood and Blumer, 1975; Hase and Hites, 1976b). In addition, the low abundance of alkylated derivatives and the similarities with the distributions obtained in combustion products of fossil fuels strongly suggested a pyrolytic origin for these complex mixtures (Hase and Hites, 1976b; Youngblood and Blumer, 1975). Hase and Hites (1976b) and Blumer and Youngblood (1975) disagree as to the type of pyrolytic source. Blumer and Youngblood suggest that forest fires could be a major source of PNAH's since the wide range of combustion temperatures in forest fires could form the more alkylated derivatives of PNAH's found in marine sediment. On the other hand, Hase and Hites (1976b) state that the distribution of PNAH's is very similar regardless of the pyrolytic source and that the distribution of alkylated derivatives reflects the differential water solubility of higher alkyl homologs vs unsubstituted species.

The high concentration of perylene, as compared to that of other PNAH's found in rapidly accreting reducing sediments off California, Africa, and British Columbia, suggested a different origin for the perylene (Aizenshtat, 1973; Aizenshtat, *et al.*, 1973, Orr and Grady, 1967). Yen *et al.*, (1961) stated that the perylene found in oil was not synthesized under the same conditions as the other PNAH's present in oil, but rather was extracted from the sediment through which the oil migrated. The precursor of the perylene found in these basins was thought

to be either from land biota (Aizenshtat, 1973) or from pigments of marine organisms (Orr and Grady, 1967). Since PNAH's are present in petroleum (Pancirov, 1974) and persist in sediments contaminated by oil spills (Vandermuelen *et al.*, 1977; Scarrat and Zitko, 1972; Blumer and Sass, 1972), the contribution of petroleum pollution as a major source of the PNAH's present in the marine environment has been analyzed. A recent report (GESAMP, 1977), however, reasons that the input of PNAH's into the sea from petroleum is greatly outweighed by other inputs, except in areas of major oil spills, since the concentration of PNAH's in oil is far less than that in other environmental contaminants (coal, atmospheric fallout, industrial sludge, domestic sewage, creosated wharf piles, and urban runoff). Furthermore, Hase and Hites (1976b) and Youngblood and Blumer (1975) reasoned that the deficiency of unsubstituted PNAH's and the prevalence of three and four carbon alkyl homologs in petroleum ruled out petroleum spillage as a major source of PNAH's in estuarine sediment. They felt that the distribution of PNAH's in the sediment more closely reflected that formed from an incomplete combustion of wood or fossil fuels.

Degradation

In the marine environment, degradation of PNAH's is primarily by biological metabolism or chemical oxidation. Chemical oxidation by ultraviolet light commonly occurs in coastal waters (McGinnes and Snoeyink, 1974), though oxidation by halogenation and ozonation could occur in waters near sewage treatment plants. The oxidation of 3,4 benzpyrene and benzanthracene to their quinones has been studied by Kuratsune and Hirohito (1962) and McGinnes and Snoeyink (1974), and reviewed by Radding *et al.*, (1976). The rate of decomposition of PNAH's in marine waters has been found to increase with UV light intensity (McGinnes and Snoeyink, 1974) and with absorption onto minerals present in suspended marine sediment, i.e., calcite (Suess, 1968) and kaolinite (McGinnes and Snoeyink, 1974). Nagata and Kondo (1977) studied the stability of PNAH's in water-acetone solutions and found that more than 90 percent of the PNAH's (chrysene, fluorene, pyrene, and 3,4 benzpyrene) remained after ten hours of radiation from a Hg lamp. This finding agreed with that of McGinnes and Snoeyink (1974) who found that a residue of 3,4 benzpyrene remained regardless of the duration of UV radiation. Suess (1968) found that twenty hours of daylight exposure degraded 90 percent benzpyrene in aqueous solution. Apparently, conditions other than light are important in determining the rate of photodegradation.

Research on the microbial degradation of PNAH's has been investigated by Gibson (1976). He and his coworkers have concentrated on the initial oxygenation reaction of PNAH's. Unlike mammalian oxidation of PNAH's to arene oxides, bacteria were found to oxidize phenanthrene, anthracene, benzo(a)pyrene, and benz(a)anthracene to a cis-dihydrodiol.

Lee (1977) examined microbial degradation of ^{14}C-labelled hydrocarbons in estuarine waters. Degradation rates were either zero or very small ($<0.012 \pm$

0.009 μg/liter day^{-1}) for PNAH's (anthracene, fluorene, 3,4-benzpyrene, benz(a)anthracene) in unpolluted and slightly oil-polluted waters. In highly oil-polluted waters, the degradation rate increased to 0.070 and 0.038 μg/liter day^{-1} for anthracene and fluorene, respectively. This rate, however, is still substantially less than that measured for other hydrocarbons. Lee also found that incubation of PNAH's with surface sediments indicated that sediment microbes are important in the degradation of these compounds.

Conclusion

In summary, research has focused on PNAH's because of their known relationship with carcinogenesis and mutagenesis. While research on the relationship between molecular structure and carcinogenic activity has been studied for many years, the distribution and source of PNAH's in the environment has only recently become the subject of considerable debate. The findings of research to date indicate that, although biosynthesis and petroleum spillage may be sources for PNAH's in localized areas, the major source of these compounds is pyrolytic. While some researchers feel that forest fires may be responsible for the ubiquitous distribution of these compounds (Blumer and Youngblood, 1975), strong evidence suggests that the combustion of fossil fuels and atmospheric transport are primarily responsible (Hase and Hites, 1976b). In the marine environment, the transport of PNAH's is limited by their low water solubilities and high tendency for adsorption onto particulate matter, and the movement of these contaminated particles is believed to be the dominant method of transport of these compounds. Although the degradation of these compounds may result from photochemical oxidation or through the microbial action, PNAH's appear to be quite persistent, especially in anaerobic sediments. Due to their carcinogenic and mutagenic activity, the extent of their distribution, and their persistence, more research on the fate and effects of PNAH's in the environment is greatly needed.

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